FUNGI (MOULD AND YEAST) PLATE COUNT

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INTRODUCTION

Mould growth on foods with its fuzzy, cotton-like and sometimes coloured appearance is familiar to everyone. Usually a mouldy or "mildewed" food is considered unfit to eat. While it is true that moulds are concerned with the spoilage of many kinds of foods, special moulds are useful in the manufacture of certain kinds of foods or ingredients of foods.

Yeasts are as difficult to define as moulds. In Henrici's "Molds, Yeasts, and Actinomycetes", yeasts are defined as true fungi whose usual and dominant growth form is unicellular. Some of the moulds in their conidial stage are like budding yeasts, and some yeasts have a mycelial stage. An example of a genus which is sometimes listed with the moulds and sometimes with the yeasts is *Geotrichum*.

Yeasts may be useful or harmful in foods. The manufacture of foods like bread, beer, wines, vinegar, and surface-ripened cheese involves yeast fermentation. Yeasts are grown for enzymes and for food. They are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups, molasses, honey, jellies, meats, wines, beer and other food.

Partial classification of Eumycetes, or true fungi, and the classification of yeasts to include genera found in foods are shown in Figs. 1 and 2 respectively.(Source : W.C. Frazier, Food Microbiology, TATA McGraw-Hill Publishing Co. Ltd., New Delhi, 1977).

APPARATUS

1. Petri dishes (ø = 90 mm, depth = 20 mm)
2. Waring blender and flasks
3. Pipettes
4. Scissors and forceps
5. Alcohol lamps
6. Alcohol (70% v/v) swabs
7. Bent glass spreader
8. Autoclave
9. Incubator
10. Weighing balance

CULTURE MEDIA* AND REAGENTS

1. Potato dextrose agar

2. Sabouraud dextrose agar

3. 0.05% agar in 0.85% saline (NaCl) solution

4. Chloramphenicol (antibiotics)

   Before autoclaving the media, weigh 50 – 100 mg of chloramphenicol and dissolve in 10 ml of 95% ethanol (medium : 1000 ml).

   * Refer to Appendix B for methods of media preparation.

PROCEDURE

Sampling procedure

Refer to Aerobic Plate Count.

Sample preparation

1. Weigh 25 g of the sample and put them into a Waring blender flask.

2. Add in 225 ml of 0.05% agar in sterile saline (0.85% NaCl).

3. Blend for about 3 min at low speed (sample 0.1 g / 1 ml : One ml of this suspension contains 0.1 g of sample i.e. a 10^{-1} dilution.

4. Pipette 10 ml of the suspension from the 10^{-1} dilution into 90 ml of 0.85% saline diluent to give a dilution of 10^{-2}.

5. Prepare further dilutions by mixing 1 ml of the well mixed diluted sample solution with 9 ml of 0.85% saline diluent.

Analytical procedure

1. Using 10^{-1} stage dilutions, pipette 0.1 ml aliquots to each of the 2 potato dextrose agar plates or the Sabouraud dextrose agar plates, for every dilution.

2. Spread gently and evenly over the surfaces of the agar plates with a sterile bent glass spreader.

3. Allow the plates to stand until the aliquot has been absorbed completely, which should be within 15 min after the spreading.

4. Invert the plates and incubate at 23° ± 2°C for 5 days (maximum for 7 days).

5. Count the plates which have 10 – 100 colonies.

6. The fungi plate count for the sample is calculated as shown below :

Table 1. Calculation of fungi plate count (Spread plate method) using 0.1 ml of 10^{-1} dilution.

Dilution	The average colony count obtained on duplicate petri dishes	Record (fungi/g)
10^{-2}	19	1.9×10^3
10^{-3}	2*	
10^{-4}	0	

* Not recorded because number of colonies is <10. As this method uses 0.1 ml of 10^{-1} dilution (0.1 g/ml) sample solution, care should be taken when the results are obtained from using 10^{-2} dilution (0.01 g/ml) as the beginning stage.

If the number of colonies obtained is <10 in 10^{-2} stage, it implies that the count is <1000/g.

If necessary the number of colonies of 1 ml of 10^{-1} (1 g/ml) are determined using 3 agar plates with 0.3 ml, 0.3 ml and 0.4 ml (total of 1 ml) of the 10^{-1} dilution or 2 agar plates with 0.5 ml each (total of 1 ml). In this case if the number of colonies obtained is <10 in the 10^{-1} dilution, it implies that the count is <100/g.

Table 2. Fungi plate count (Pour plate method) using 1 ml of 10^{-1} dilution.

Dilution	The average colony count obtained on duplicate petri dishes	Record (fungi/g)
10^{-1}	19	1.9×10^2
10^{-2}	2	
10^{-3}	0	

If the number of colonies obtained is <10 in 10^{-1} dilution, it implies that the count is <100/g.

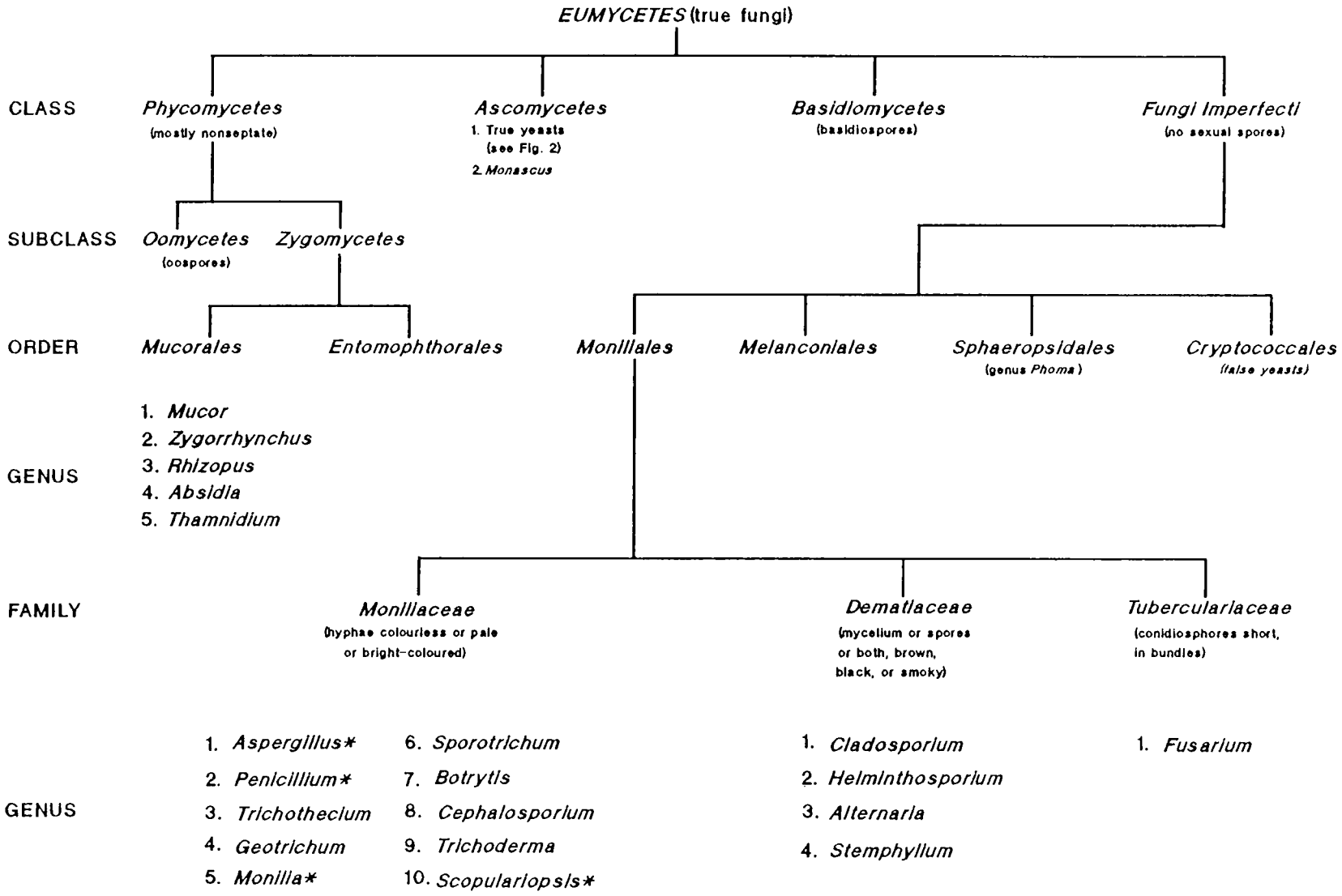
7. Mould and yeast plate count.

Judgement is based on the shape of colonies and the counts of mould and yeast respectively.

REFERENCES

Shokuhin Eisei Kensa Shishin, Guide to Food Hygiene Examination (authorized by the Ministry of Health and Welfare), Japan Food Hygiene Association. (1990).

Frazier, W. C. (1977). Food Microbiology, TATA McGraw-Hill Publishing Co. Ltd., New Delhi.



* Perfect species in Ascomycetes

Fig. 1. Partial classification of *Eumycetes*, or true fungi

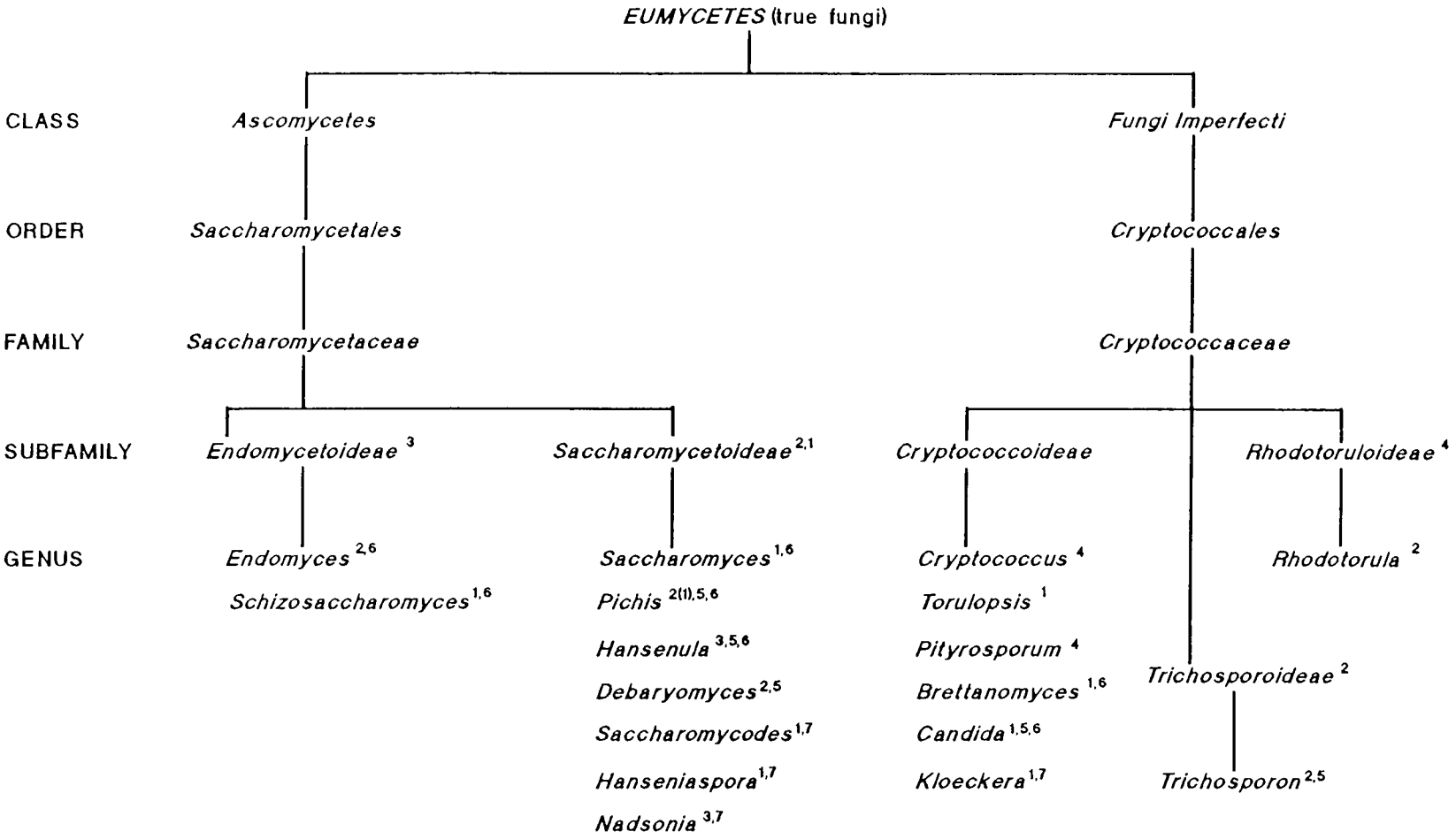


Fig. 2. Classification of yeasts to include genera found in foods.

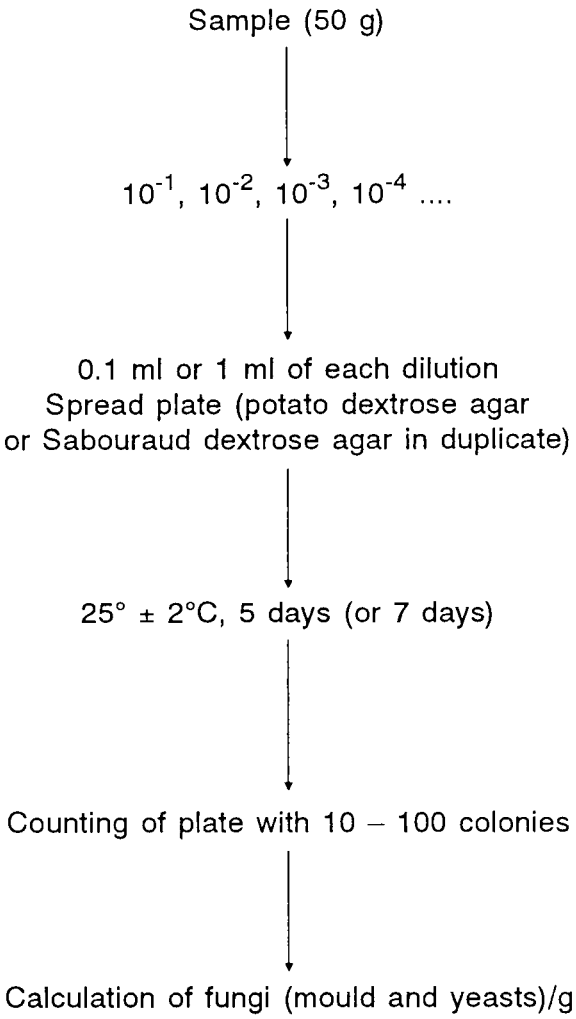


Fig. 3. Flow diagram of the procedure for Fungi Plate Count